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G Praveen Kumar,
Assistant Professor (Contractual)
Department of Fish Processing Technology, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur, Nellore, Andhra Pradesh, India

Dr. G Vidya Sagar Reddy
Senior Scientist and Head
Fisheries Research Station, Palair, Khamman, Telangana, India

Dr. K Dhanapal
Professor and University Head
Dept. of Fish Processing Technology, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur, Nellore, Andhra Pradesh, India

P Hari Babu
Principal Scientist
Livestock Research Station, Lam Farm, Guntur, Andhra Pradesh, India

Corresponding Author:
G Praveen Kumar,
Assistant Professor (Contractual)
Department of Fish Processing Technology, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur, Nellore, Andhra Pradesh, India

Effect of frozen storage on the quality and shelflife of mrigal (*Cirrhinus mrigala*)

G Praveen Kumar, GVS Reddy, K Dhanapal and P Hari Babu

Abstract
Indian major carps contribute to the major share in the aquaculture of different fish species in India. Mirgal is one among the Indian Major Carps. In the present study, the quality of mrigal was evaluated during frozen storage to estimate the shelflife. In order to achieve the objective the changes in proximate composition, physicochemical parameters, microbial quality and sensory analysis were conducted. From the results it was observed that during frozen storage, the moisture content increased and the crude protein, lipid and ash contents decreased continuously. The p^H value of fish increased initially up to 60^{th} day of frozen storage and then decreased till the end of storage. TMA-N and TVB-N showed an increasing trend throughout the storage period. The water holding capacity decreased continuously with increase in storage period. The lipid freshness parameters (PV, TBA and FFA) increased during the entire period of storage. The microbial load decreased with increase in storage time. During frozen storage, TPC counts decreased while the psychrophilic counts increased. From the results it was concluded that mrigal had a shelflife of 165 days during frozen storage.

Keywords: mrigal, frozen storage, quality, shelflife

Introduction
Fish is one of the most important source of animal protein available in the tropics and has been widely accepted as good source of quality protein and other elements for maintenance of healthy body. Generally, fish is considered as good source of vitamins B_{12} and B_6 as well as flourine and iodine which are needed for development of strong teeth and for prevention of goiter in human [1]. On a global scale, fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture [2, 3]. Indian Major Carps are the second major species produced from aquaculture next to silver and big head carps [4]. In India, Andhra Pradesh ranks second in total fresh water fish production. Mrigal (*Cirrhinus mrigala*), a carp endemic to Indo-Gangetic riverine systems is one of the three Indian major carp species cultivated widely in Southeast Asian countries.
The loss of quality depends directly on the nature of the fish species and the storage conditions [5]. In the recent times, modern biotechnology has introduced new techniques that can detect early fish contamination, improve the taste, modify the quality of fish and prolong the shelf life and also impart disease resistance to the fish [6].
Freezing is used for long-term storage. The fishes are cooled below temperatures of – 40 °C and stored at -18 °C. The much long shelf life is due to almost complete halting of autolytic and bacterial action at these lower temperatures and also free water is effectively locked as ice [7]. Freezing may be useful for long-term storage and export through cold chain. Based on the above knowledge, a study was conducted to study the effect of freezing on the quality and shelf life of mrigal.

Materials and Methods
Mrigal (*Cirrhinus mrigala)* harvested from nearby farmer's ponds located at Muthukur, S.P.S.R. Nellore District, Andhra Pradesh were brought to the Fish Processing laboratory, College of Fishery Science, Muthukur in an plastic polystyrene insulated containers (without ice) within 10 minutes of harvest and used for frozen storage studies. The average length and weight of fish were 39.36 ± 0.25cm and 894.50 ± 24.14 grams, respectively.
Immediately after reaching the laboratory, the fish were washed with ice cold potable water. After washing, fishes were randomly drawn for sampling to evaluate proximate, biochemical and microbiological parameters to evaluate the values for fresh fish.

The remaining whole lot was subjected to freezing at -40 ˚C for 6 hrs in a freezer unit. After completion of freezing, the frozen fishes were packed in a 400 gauze high density polyethylene (HDPE) covers in the proportion of two fishes in each cover and immediately transferred to a cold storage unit. The cold storage temperature was maintained at a temperature of -18 to -20 ˚C for further studies. Initially after completion of freezing process, three fishes were used for estimating various quality parameters and one fish after thawing was used for sensory assessment to study the effect of freezing. Subsequently, samples were randomly drawn for sampling with sampling at regular interval of 15 days and the quality changes during frozen storage were determined.

Proximate composition of mrigal was analyzed by the method described in AOAC [8]. TVBN and TMAN was determined by the Conway method [9] and expressed as mg/100 g of meat. Peroxide value was determined using method adopted by Jacobs [10]. TBA value was determined as described by Tarladgis [11] and expressed as mg malonaldehyde per kg of fish sample. Free fatty acid was estimated by Olley and Lovern [12] method. Water holding capacity (WHC) of fish muscle was measured by modified centrifugation method described by Delvalle and Gonzales-Inigo [13] and expressed in percentage (%). The microbiological analysis were carried out following the methods described by APHA [14]. This study included Total Plate Count (TPC), *Staphylococcus aureus*, *Escherichia coli*, *Faecal streptococci*, total *Psychrophiles*, Total H_2S producers, *Aeromonas* spp, *Pseudomonas* spp, *Vibrio* spp, *Salmonella* spp. and *Listeria monocytogenes*.

Fish were observed for changes in their appearance, odour, taste and texture by 8 Panel members. The sensory evaluation for overall acceptability was carried out after cooking the selected fish and it was done by 8 trained panelists using 9 point hedonic scales viz., Like extremely (9), Like very much (8), Like moderately(7), Like slightly(6), Neither like nor dislike (5), Dislike slightly (4), Dislike moderately (3), Dislike very much (2), Dislike extremely (1) Torry meter readings were taken to know the correlation between sensory score and instrumental score.

Statistical Analysis

The SPSS 16 (IBM, 2010) Statistical Package for Social Sciences was used for analysis of the experimental results. Sufficient numbers of samples were carried out for each analysis. The results were expressed as mean ± standard deviation (SD). Correlations were established between the various characteristics by using “Post Doc” coefficient of SPSS (IBM, 2010). Sensory scores for overall acceptance of the product were correlated with the storage time, and the shelf life of mrigal during frozen storage was calculated using linear regression plot.

Results and Discussion

Changes in proximate composition during frozen storage

During frozen storage, moisture content increased with increase in storage period. The moisture content for fresh fish was 76.31 ± 0.80%. The moisture content increased immediately after freezing and during subsequent storage. It increased from 76.79 ± 0.66% to 84.87 ± 0.49% at the end of 150 days frozen storage period (Table 1). During freezing and frozen storage, moisture content increased steadily with increase in storage period. Asgharzadeh *et al*. [15] reported that the carp muscle maintained a higher moisture values throughout the storage period for 6 months. Increase in expressible moisture was observed by Natseba *et al*. [16] in frozen Nile perch.

The lipid content decreased with increase in frozen storage period. The initial fat content of fresh mrigal fish was 7.04 ± 0.10%. The lipid content decreased immediately after freezing and during frozen storage from 6.84 ± 0.06% to 2.81 ±0.07% up to 150 days (Table 1). Changes in lipids occur through hydrolysis and oxidation mechanism. In the present study, a steady decrease in the lipid content was noticed during the entire frozen storage period. The changes in fat content during frozen storage could be associated with the oxidation of fat. Generally, an inverse relationship exists between the total lipid and moisture content in fish. These results are in accordance with Raju *et al*. [17] in silver carp, Keyvan *et al*. [18] in *Rutilus frisi kutum* fish where the changes in fat content during frozen storage could be associated with the oxidation of fat. Meenakshi *et al*. [19] also reported similar results in *Cyprinus carpio*. A decreasing trend in lipid content was observed by Mazorra-Manzano [20]. Considering the low fat content, it can be assumed that lipid hydrolysis and oxidation would have little impact in the overall quality of fish during the storage period.

The protein content in fresh mrigal meat was initially 14.89 ± 0.76%. During freezing and frozen storage, the protein content decreased from 14.65 ± 0.72% to 11.34 ± 0.43% (Table 1). In the present study, a steady decrease in total protein content was observed with increase in storage time till the end of the storage period. This could be expected, as some proteins and other non-protein nitrogenous matter are lost in the free drip. Raju *et al*. [17] reported that the total protein content decreased during frozen storage of silver carp mainly due to the loss of some soluble proteins and other non-protein nitrogenous matter during thawing process as free drip. A similar decrease in total protein has been observed by Keyvan *et al*. [18] in *Rutilus frisi kutum* and Meenakshi [19] in *Cyprinus carpio*.

The ash content of frozen mrigal fish decreased during the entire frozen storage period. During frozen storage, the ash content ranged between 1.72 ± 0.02% (0 hour) to 0.95 ± 0.02% (24th hour) (Table 1).

Table 1: Changes in proximate composition of mrigal during frozen temperature.

Storage period (days)	Moisture* (%)	Protein* (%)	Lipid* (%)	Ash* (%)
Fresh	76.31 ± 0.80^{a}	14.89 ± 0.76^{e}	7.04 ± 0.10^{h}	1.76 ± 0.09^{f}
1 (immediately after freezing)	76.79 ± 0.66^{ab}	14.65 ± 0.72^{e}	6.84 ± 0.06^{gh}	1.72 ± 0.02^{f}
15	77.72 ± 0.58^{ab}	14.25 ± 0.63^{de}	6.45 ± 0.09^{g}	1.57 ± 0.08^{ef}
30	78.44 ± 0.74^{bc}	13.74 ± 0.65^{cde}	6.37 ± 0.20^{g}	1.42 ± 0.13^{de}
45	79.92 ± 0.87^{cd}	13.47 ± 0.51^{bcde}	5.17 ± 0.40^{f}	1.42 ± 0.07^{de}
60	80.65 ± 0.61^{de}	13.21 ± 0.58^{bcde}	4.78 ± 0.11^{ef}	1.35 ±0.0.06^{cd}
75	81.49 ± 0.78^{d}	12.89 ± 0.51^{abcd}	4.37 ± 0.11^{de}	1.24 ± 0.04^{bcd}
90	82.16 ± 0.40^{cd}	12.62 ± 0.47^{abcd}	4.03 ± 0.14^{d}	1.17 ± 0.06^{bc}
105	82.60 ± 0.53^{fg}	12.40 ± 0.41^{abc}	3.83 ± 0.1^{cd}	1.16 ± 0.51^{bc}

120	83.37 ± 0.47^{fgh}	12.10 ± 0.36^{abc}	3.42 ± 0.17^{bc}	1.13 ± 0.04^{ab}
135	84.06 ± 0.37^{gh}	11.80 ± 0.37^{ab}	3.08 ± 0.08 ^{ab}	1.05 ± 0.04^{ab}
150	84.87 ± 0.49^{h}	11.34 ± 0.43^{a}	2.81 ± 0.07^{a}	0.95 ± 0.02^{a}

*Each value is represented as arithmetic mean ± SD of n =3.
^{abcdefg} Means followed by the same superscript with in a column are not significantly different (p>0.01).

Changes in Physicochemical parameters during frozen storage

The p^{H} of fresh fish meat was 6.78 ± 0.03. After freezing and during storage, the pH values showed a decreasing trend till 60th day (6.22 ± 0.04) and later increased with increase in storage period to 6.83 ± 0.01 on 150th day of storage period (Table 2). The initial increase and decline in value of pH may be correlated with changes in total viable count. Ben-gigirey *et al*. [21] reported an increase in pH during frozen storage of albacore tuna at -18 °C and -25 °C. These results are similar to the observations made by Raju *et al*. [17] in silver carp where the value of pH decreased during the initial 30 days followed by significant increase during later part of the storage due to production of volatile base compounds, as indicated by the increase in total plate count. Similar results were obtained by Tokur *et al*. [22] in trout, Orak and Kayisoglu [23] in *Gadus* spp., *Mugil* spp. and *Engraulis* spp. and Obemeata *et al*. [24] in tilapia stored at -18 ^{0}C where in the decrease in p^{H} is attributed to the fact that fermentation of carbohydrate to acid.

The TVBN content of fresh sample was 0.16 ± 0.00 mg/100 g of meat. After freezing and during frozen storage period, the values increased from 0.38 ± 0.08 mg/100g of meat to 3.11 ± 0.00 mg/100g of meat (Table 2). Volatile base nitrogen indicates the production of ammonia, mono-di and trimethylamine nitrogen and are found in the common pattern of spoilage. Increase in the content of TVB-N significantly correlated with the decrease in overall acceptability scores in the present study (P < 0.01). Ben-gigirey *et al*. [21] reported an increase in TVBN content of frozen albacore tuna stored at -25 °C for 12 months. Similar results were obtained by Chakrabarti [25] in Indian major carps, Magnússon and Martlnsdóttlr [26] in cod and perch fillets, Sarma *et al*. [27] in pink perch and oil sardine, Raju *et al*. [17] and Asgharzadeh *et al*. [15] in silver carp, Orak and Kayisoglu [23] in *Gadus euxinus, Mugil cephalus* and *Encrasicholus.*

Estimation of TMA-N is generally recommended for assessing the quality of frozen fish. During frozen storage, the TMAN content increased from 0.16 ± 0.00 mg/100g of meat to 2.83 ± 0.00 mg/100g of sample after 150 days of storage (Table 2). The low TMA-N levels recorded in the present study could be related to the composition of the microbial flora and the relatively low pH values encountered during storage. The optimum p^{H} for activity of the bacterial TMAO reducing enzymes has been reported to be 7.2-7.4 [28, 29]. Similar results were recorded by Sarma *et al*. [27] in pink perch and Orak and Kayisoglu[23] in *Gadus Euxinus, Mugil cephalus* and *Encrasicholus.*

Table 2: Changes in physic-chemical parameters of mrigal during frozen storage.

Storage period (days)	p^{H} value	PV * (meq/kg of fat)	TBA value * (mg of MA/kg of sample)	FFA * (% of oleic acid)	TVBN* (mg/100g of meat)	TMA* (mg/100g of meat)	WHC *
Fresh fish	6.78 ± 0.03 ^{efg}	4.00 ± 0.28 ^{a}	0.21 ± 0.04 ^{a}	0.0007 ± 0.00 ^{a}	0.16 ± 0.00 ^{a}	0.08 ± 0.01 ^{a}	95.09 ± 0.38 ^{f}
1$	6.72 ± 0.01 ^{ef}	6.00 ± 0.00 ^{b}	0.36 ± 0.05 ^{a}	0.0011± 0.00 ^{b}	0.38 ± 0.08 ^{a}	0.16 ± 0.00 ^{a}	86.47 ± 1.44^{e}
15	6.68 ± 0.00 ^{de}	7.40 ± 0.28^{b}	0.50 ± 0.06 ^{ab}	0.0011 ± 0.00 ^{b}	0.62 ± 0.01 ^{b}	0.28 ± 0.02 ^{a}	84.62 ± 1.64e
30	6.60 ± 0.00 ^{cd}	10.60 ± 0.20 ^{c}	0.69 ± 0.03 ^{bc}	0.0014 ± 0.00 ^{c}	0.92 ± 0.02 ^{c}	0.63 ± 0.03^{b}	80.81 ± 1.01 ^{d}
45	6.54 ± 0.01 ^{c}	12.20 ± 0.20 ^{d}	0.84 ± 0.04 ^{c}	0.0017 ± 0.00 ^{d}	1.39 ± 0.11 ^{d}	0.99 ± 0.02 ^{c}	78.27 ± 1.56 ^{cd}
60	6.22 ± 0.04 ^{a}	14.40 ± 0.02 ^{e}	0.94 ± 0.00 ^{cd}	0.0026 ± 0.00 ^{e}	1.80 ± 0.16 ^{e}	1.70 ± 0.17 ^{d}	78.11 ± 0.63 ^{cd}
75	6.26 ± 0.05a	16.53 ± 0.38^{f}	1.20 ± 0.09 ^{d}	0.0029 ± 0.00^{f}	2.21 ± 0.02 ^{f}	1.97 ± 0.01 ^{e}	77.18 ± 0.61 ^{bc}
90	6.39 ± 0.07^{b}	18.93 ± 0.75^{g}	1.59 ± 0.03 ^{e}	0.0029 ± 0.08 ^{f}	2.37 ± 0.01 ^{f}	2.1 ± 0.13 ^{e}	77.17 ± 1.27 ^{bc}
105	6.51 ± 0.01c	20.00 ± 0.65^{g}	1.90 ± 0.01 ^{f}	0.0030 ± 0.00^{fg}	2.60 ± 0.10 ^{g}	2.31 ± 0.01 ^{f}	75.46 ± 0.79 ^{bc}
120	6.73 ± 0.04^{ef}	22.66 ± 1.00^{h}	2.26 ± 0.06 ^{g}	0.0031 ± 0.00^{g}	2.94 ± 0.11 ^{h}	2.66 ± 0.06 ^{g}	74.86 ± 0.37 ^{b}
135	6.80 ± 0.01^{fg}	27.80 ± 0.28i	2.66 ± 0.12 ^{h}	0.0033 ± 0.00^{h}	3.09 ± 0.00 ^{h}	2.81 ± 0.00 ^{g}	74.12 ± 0.10 ^{b}
150	6.83 ± 0.01^{g}	30.20 ± 0.74^{j}	3.33 ± 0.27 ^{i}	0.0036 ± 0.00^{i}	3.11 ± 0.00 ^{h}	2.83 ± 0.00 ^{g}	71.16 ± 0.65 ^{a}

* Each value is represented as arithmetic mean ± SD of n =3.
^{abcdefg} Means followed by the same superscript with in a column are not significantly different (p>0.01).
$ - immediately after freezing

The FFA of fresh mrigal was 0.0007± 0.00% of oleic acid. After freezing and during frozen storage, the value increased from 0.0011 ± 0.00% of oleic acid to 0.0036 ± 0.00% of oleic acid at the end of 150 days (Table 2). FFA, resulting from lipid hydrolysis accumulates during frozen storage and accelerates quality deterioration [30]. In the present study, hydrolysis of fish lipids in frozen condition increased gradually during storage, forming a good linear correlation. Ben-gigirey *et al*. [21] reported an increase in FFA content of frozen albacore tuna till 9 months stored at -18 °C. Rodríguez *et al*. [31] reported an increase in FFA content of coho salmon till 15 months of frozen storage. Similar results were obtained by Srikar [32] in Pink perch and Sarma *et al*. [27] in Pink perch where the lower increase in FFA may be attributed to the slower rate of lipid oxidation. Similar observations have been recorded for other species. Similar observation was made by Aubourg and Medina [33] in horse mackerel and Stodolnik *et al*. [34] in *Scomber scombrus*. Sequeira-Munoz *et al*. [35] showed an increase in the content of FFA of lipids extracted from frozen carp fillets frozen stored up to 75 days. Aranda *et al*. [36] also found that free fatty acids increased linearly with the length of time of storage at -18 ^{0}C in frozen jack mackerel stored for 120 days. Similar results were observed by Keyvan *et al*. [18] in *Rutilus frisi kutum* fish and Makri [37] in gilthead seabream (*Sparus aurta*). The result of the present study implies that lipolytic enzymes were active in the muscle of frozen fish throughout the storage period.

The TBA of fresh mrigal was 0.21± 0.04 mg of malonaldehyde/kg of meat. After freezing and during frozen storage, the value increased from 0.36 ± 0.05 mg of

malonaldehyde/kg to 3.33 ± 0.27 mg of malonaldehyde/kg at the end of 150 days storage period (Table 2). Increase in TBA values during frozen storage has been noticed in the present study throughout the period. These results are in agreement with the observations of Aubourg and Medina [33] in frozen stored cod and haddock and observed that the increase in TBA values is comparatively less at -30 ^{0}C than -10 ^{0}C. Rodríguez *et al*. [31] reported an increase in TBA content of coho salmon during frozen storage till 15 months. Similar results were also observed by Keyvan *et al*. [18] in *Rutilus frisi kutum fish*, Orak and Kayisoglu [23] in *Gadus Euxinus, Mugil cephalus* and *Encrasicholus* in three different styles (like whole, gutted and filleted). In gilthead sea bream Makri [36] observed an increase in values of TBA till 340 days of frozen storage and then decreased as the TBA reactive substances were prone to interact with biological constitutes present in muscle leading to decrease. Asgharzadeh *et al*. [15] revealed similar results in silver carp.

The water holding capacity decreased from 95.09 ± 0.38% (0 day) to 71.16 ± 0.65% at the end of storage study and the results are included in Table 2. The water holding capacity decreased throughout the present frozen storage study for 150 days. Asgharzadeh *et al*. [15] reported that the expressible moisture content of unwashed silver carp mince increased considerably during the frozen storage time. This indicated that less water was imbibed in the gel matrix as a result of a protein denaturation increase by extended frozen storage leading to a lower water affinity and accordingly, WHC decreases [38, 39]. The total losses during thawing and pressing were taken as a measure of the water holding capacity (WHC) of the fresh and frozen scallop meats [37]. Decrease in water holding capacity may be caused by ice crystal growth and protein and lipid oxidation [40].

Changes in microbial quality during frozen storage of mrigal

The total plate count count in fresh mrigal fish was 8.36 x 10^2 cfu/gram of meat. During freezing and frozen storage study, the total bacterial count increased upto 30th day 15.00 x 10^2 cfu/gram of meat and then decreased till the end of frozen storage period 2. 24 x 10^2 cfu/gram of meat (Table 3). The total plate count increased during frozen storage up to 30th day and later decreased during further period of frozen storage. Nickelson *et al*. [41] observed a similar trend in the black drum, sand trout and tilapia. The increase in TPC was due to the utilization of NPN matter during storage [42, 43]. The same can be speculated in the present study also.

The number of psychrophiles in fresh mrigal fish were estimated to be 2.98 x 10^2 cfu/gram of meat. During freezing and frozen storage study, the *psychrophilic* count increased from 3.54 x 10^2 cfu/gram of meat to 1.09 x 10^3 cfu/gram of meat at the end of frozen storage period (Table 3). In case of *psychrophiles*, the count increased continuously. On the contrary, the sulphur producing bacterial counts were not detectable during storage.

The *Staphylococcus aureus* count was estimated to be less than 1.0 x 10^1 cfu/g of meat in the samples throughout the storage period. *Escherichia coli* and *Faecal streptococci* were estimated and the value is less than 1.0 x 10^1 cfu/g of meat in the samples throughout the storage period. *Aeromonas* spp. were absent both in fresh fish and frozen fish throughout the storage period. *Pseudomonas* spp. was estimated to be less than 1.0 x 10^1 cfu/g of meat in the samples. Sulphur producing bacteria was estimated to be less than 1.0 x 10^1 cfu/g of meat in the samples. Pathogenic bacteria like *Vibrio* spp., *Salmonella* spp. and *Listeria monocytogenes* were absent in the fresh fish and frozen fish.

Table 3: Changes in TPC and *Psychrophilic* counts during frozen storage of mrigal.

Storage period (days)	Total plate count* (cfu/gram of meat)		*Psychrophiles* * (cfu/gram of meat))	
	24 hrs#	48 hrs#	24 hrs#	48 hrs#
Fresh fish	8.36x 10^2 (2.92)	8.45 x 10^2 (2.93)	2.98 x 10^2 (2.47)	3.10 x 10^2 (2.49)
1$	10.70 x 10^2 (3.02)	11.00 x 10^2 (3.04)	3.54 x 10^2 (2.55)	3.62 x 10^2 (2.56)
15	11.5 x 10^2 (3.06)	11.85 x 10^2 (3.07)	5.56x 10^2 (2.75)	5.60x 10^2 (2.75)
30	15.00 x 10^2 (3.17)	15.60 x 10^2 (3.19)	6.20 x 10^2 (2.79)	6.30 x 10^2 (2.80)
45	6.84 x 10^2 (2.84)	7.04 x 10^2 (2.85)	6.84 x 10^2 (2.84)	7.00 x 10^2 (2.85)
60	5.60x 10^2 (2.75)	5.83 x 10^2 (2.77)	7.58 x 10^2 (2.88)	7.64 x 10^2 (2.88)
75	5.10 x 10^2 (2.71)	5.27 x 10^2 (2.72)	8.60 x 10^2 (2.93)	8.70 x 10^2 (2.94)
90	4.02 x 10^2 (2.60)	4.10 x 10^2 (2.61)	9.36 x 10^2 (2.97)	9.50 x 10^2 (2.978)
105	3.32 x 10^2 (2.52)	3.40 x 10^2 (2.53)	9.82 x 10^2 (2.99)	1.00 x 10^3 (3.00)
120	2.68x 10^2 (2.43)	2.74 x 10^2 (2.43)	1.01x 10^3 (3.00)	1.02 x 10^3 (3.01)
135	2.68x 10^2 (2.43)	2.83 x 10^2 (2.45)	1.06 x 10^3 (3.02)	1.74 x 10^3 (3.03)
150	2.24x 10^2 (2.35)	2.37x 10^2 (2.37)	1.09x 10^3 (3.03)	1.09 x 10^3 (3.04)

* Each value is represented as arithmetic mean of 2 estimates.
# Period of incubation,
Figures in parenthesis indicate Log bacterial counts
cfu = colony forming units

Changes in sensory quality during frozen storage

The overall sensory scores decreased during the frozen storage study. The sensory score allotted for fresh fish was 8.94 ± 0.05. After freezing and during frozen storage the overall sensory scores decreased from 8.58 ± 0.10 to 5.20 ± 0.24 (Table 4). A decrease in organoleptic scores was noticed throughout the period of frozen storage. The sensory evaluation data for frozen stored fish showed a high negative correlation with storage period. Correlation between mean panel scores for acceptance and storage period was significant at 0.01 level. Similar results were obtained by Orak and Kayisoglu [23] in *Gadus euxinus*, *Mugil cephalus* and *Encrasicholus.*

Table 4: Changes in the Overall Sensory Score (OSS) during frozen storage of mrigal.

Storage period (days)	OSS*
Fresh fish	8.94 ± 0.05^{g}
1	8.58 ± 0.10 ^{g}
15	8.15 ± 0.12^{f}
30	7.90 ± 0.20 ^{f}
45	7.50 ± 0.15 ^{e}
60	7.15 ± 0.12^{e}
75	6.75 ± 0.31^{d}
90	6.50 ± 0.27 ^{cd}
105	6.10 ± 0.12^{bc}
120	5.85 ± 0.12 ^{b}
135	5.60 ± 0.12^{a}
150	5.20 ± 0.24 ^{a}

* Each value is represented as arithmetic mean ± SD of n =8.

^{abcdefg} Means followed by the same superscript with in a column are not significantly different (p>0.01)

Conclusion

From the results it was observed that the moisture content increased and the crude protein, lipid and ash contents decreased continuously. The p^{H} value of fish increased initially up to 60th day of frozen storage and then decreased till the end of storage. The biochemical parameters like TMA-N, TVB-N, TBA and FFA has showed an increasing trend throughout the storage period whereas the water holding capacity decreased continuously with increase in storage period. During frozen storage, TPC counts decreased while the psychrophilic counts increased. The overall acceptability of the frozen stored fish decreased with increase in storage period. On correlating the overall acceptable sensory scores with storage time, the fish was acceptable for 165 days at frozen storage.

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